

## Poster Session I

lethal GVHD while maintaining GVL activity after BMT with allogeneic naive (uncultured) thymidine kinase (TK)-expressing transgenic T cells. However, the efficacy of GCV control of GVHD and its impact on GVL activity following BMT with ex vivo activated, TK transduced and selected (Td) donor T cells has not been thoroughly characterized because of the significantly reduced GVHD-inducing potential of cultured cells compared to naive T cells in both preclinical models and initial clinical trials. In these experiments, we used A20 tumor cells (B cell lymphoma of BALB/c origin) that were modified to stably express a click beetle red luciferase-EGFP fusion reporter gene (A20-CBRluc/egfp) and monitored disease progression in vivo using optical bioluminescence imaging (BLI). To induce leukemia, BALB/c recipients were lethally irradiated and reconstituted with C57BL/6 BM supplemented with or without A20-CBRluc/egfp leukemia cells. Mice receiving leukemia cells were then left untreated or injected with  $4 \times 10^6$   $\Delta$ CD34-TK (chimeric fusion suicide gene) Td donor T cells the day of BMT. Ten of ten BALB/c mice transplanted with BM and A20-CBRluc/egfp cells exhibited tumor engraftment and growth, with 8 mice dying from leukemia before day 35. In contrast, we observed no significant tumor signal in the majority (92%) of untreated (No GCV) mice that received  $\Delta$ CD34-TK Td T cells. However, these animals developed GVHD with high mortality ( $P = .003$  compared to BM only control) and significant weight loss ( $P < .05$  from day 10 onward compared to BM only control). Although mice treated with GCV from days 4–10 after BMT were protected from GVHD, 50% of these animals developed leukemia. In contrast, none of the mice developed leukemia if GCV treatment was delayed until day 10 after BMT. Importantly, this GVL effect was obtained in the absence of GVHD, as evidenced by the improved survival (88% survival at day 60 post-BMT) and weight gain of the day 10–16 GCV treated mice compared to the untreated (No GCV) control. These data suggest that donor T cell dependent GVL effects against A20 cells can be preserved while the severity of GVHD is reduced, further demonstrating the potential of the HSV-TK/GCV system to reduce acute GVHD without impairing GVL activity.

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**"MINI" METHOTREXATE AND FK506 AS GVHD PROPHYLAXIS IN PEDIATRIC UMBILICAL CORD BLOOD TRANSPLANTATION**

Staba, S.L., Hunger, S.P., Wingard, J.R. University of Florida, Gainesville, FL.

A majority of umbilical cord blood transplants to date utilize steroids and cyclosporine for graft-versus-host disease (GVHD) prophylaxis. Steroids are associated with multiple complications including hyperglycemia, hypertension, avascular necrosis/osteoporosis, and significant risk of infection. In an effort to decrease the adverse effects of GVHD prophylaxis, we chose an alternative combination of "mini" methotrexate ( $5 \text{ mg/m}^2 \times 4$  doses) and FK506. Here we report our experience with this GVHD prophylaxis regimen. Ten children (ages 4 months to 13 years) underwent umbilical cord blood transplant at the University of Florida between June 2004 and June 2005. All patients received a cord blood unit that was matched at 6/6 ( $n = 4$ ), 5/6 ( $n = 2$ ), or 4/6 ( $n = 4$ ) HLA loci. Children with malignant disease received either Cyclophosphamide/TBI or Etoposide/TBI as the preparative regimen, while children with non-malignant disease received Busulfan, Cyclophosphamide, and ATG. Umbilical cord blood cell dose ranged from  $3.4\text{--}23.7$  (median  $7.8$ )  $\times 10^7$  nucleated cells/kg. All children received Methotrexate  $5 \text{ mg/m}^2$  on days 1, 3, 6, and 11, with Leucovorin on days 4, 7, and 12. No doses of Methotrexate were held. FK506 was started as a continuous infusion at day  $-3$ , targeting levels of 10–15. The FK506 was changed to twice daily oral dosing when tolerated. **Results:** 8 of the 10 children engrafted, with engraftment occurring at a median of 15 (range 13–38) days post transplant. Rates of acute and chronic GVHD were low, with no grade III or IV acute GVHD seen, and no extensive chronic GVHD occurring. Two children had grade II acute GVHD (skin and GI). Two children developed limited chronic GVHD of the skin while weaning FK506. GVHD resolved completely in these patients with an increase in FK506 and were

subsequently weaned off immunosuppression. Mucositis was very similar to other GVHD regimens. One child became CMV PCR positive, with CMV on bronchoscopy, however had mild disease (never required oxygen) and recovered completely. There were otherwise no serious infections in this patient population beyond the time of engraftment. FK506 and "mini" Methotrexate for GVHD prophylaxis in pediatric patients undergoing umbilical cord blood transplant is well tolerated. Engraftment was not delayed and mucositis was not severe. Low rates of GVHD were seen with minimal serious infections, making this an attractive option for GVHD prophylaxis.

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**SUCCESSFUL TREATMENT OF REFRACTORY GVHD IN AN INFANT WITH MALIGNANT OSTEOPETROSIS WITH A HUMAN ADULT MESENCHYMAL STEM CELL BASED DRUG**

Kleiner, G.I.<sup>1</sup>, Gordon, P.<sup>1</sup>, Kuluz, J.<sup>1</sup>, McLaughlin, G.<sup>1</sup>, Pabwa, R.<sup>2</sup>, Ricordi, C.<sup>2</sup>, Kenyon, N.<sup>2</sup>, Monroy, R.<sup>3</sup> 1. University of Miami School of Medicine, Miami, FL; 2. Diabetes Research Institute, Miami, FL; 3. Osiris Therapeutics, Baltimore, MD.

It has been suggested that adult human mesenchymal stem cells (hMSCs) have a potential to treat graft-versus-host Disease (GVHD). At the present time, Osiris Therapeutics is in clinical development with Prochymal<sup>TM</sup>, a cellular therapy consisting of allogeneic bone marrow derived adult hMSCs for the treatment of acute gastrointestinal GVHD. The present work reports a successful treatment of an 8-month-old infant with grade III skin and gut GVHD using Prochymal<sup>TM</sup>. The infant was born with hypocalcemia with normal phosphorus and carbonic anhydrase II, and elevated PTH. Skeletal survey and bone marrow biopsy were consistent with osteopetrosis. The infant received a 4/6 unrelated cord blood transplant after standard Busulfan/Cyclophosphamide/ATG conditioning. The first graft failed and the patient developed autologous reconstitution. A second transplant (4 of 6 matched cord blood) was given at 5 months of life after Fludarabine/Cyclophosphamide/ATG conditioning regimen. The patient developed severe acute grade III skin GVHD with gastrointestinal involvement in the form of bloody diarrhea that was resistant to high dose steroids, Tacrolimus, Cellcept, and anti-CD25 antibody. A compassionate use protocol was developed for the treatment of the patient with Prochymal<sup>TM</sup>. Two intravenous infusions of Prochymal<sup>TM</sup> were given at a dose of  $8 \times 10^6$  cells/kg body weight each 3 days apart starting on day 100 post transplant. Both infusions were well tolerated with no associated toxicity or reaction. By the fourth day of treatment, there were initial signs of improvement of the skin. By day 7, the skin improvement was dramatic and episodes of diarrhea had ceased. By day 14, the resolution of this patient's refractory GVHD was essentially complete. Grade IV skin GVHD recurred approximately 1 month following initial treatment after weaning of immune suppression. Repeat treatment with Prochymal<sup>TM</sup> was administered starting on day 120 post transplant and GVHD symptoms diminished within 1 week. The patient developed 100% donor chimerism. The patient's myeloid engrafted improved from 8% on day 105 post transplant to 100% by day 145. Data suggest that MSCs suppressed the adverse immunological response, healed damaged skin and gut tissue, and aided in engraftment. We conclude that Prochymal<sup>TM</sup> may be clinically useful for treatment of refractory cases of GVHD.

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**SERUM LEVELS OF SOLUBLE INTERLEUKIN-2 RECEPTOR IS A POWERFUL MARKER OF ACUTE GRAFT-VERSUS HOST DISEASE AFTER HLA-HAPLOIDENTICAL BONE MARROW TRANSPLANTATION**

Kaida, K.<sup>1</sup>, Ikegame, K.<sup>1</sup>, Fujioka, T.<sup>1</sup>, Taniguchi, Y.<sup>1</sup>, Yoshibara, S.<sup>1</sup>, Karvakami, M.<sup>1</sup>, Hasei, H.<sup>1</sup>, Nishida, S.<sup>1</sup>, Masuda, T.<sup>2</sup>, Murakami, M.<sup>3</sup>, Kim, E.H.<sup>2</sup>, Tsuboi, A.<sup>1</sup>, Oji, Y.<sup>1</sup>, Oka, Y.<sup>1</sup>, Shirakata, T.<sup>1</sup>, Kanakura, Y.<sup>1</sup>, Ogawa, H.<sup>1</sup> 1. Osaka University Graduate School of Medicine, Suita, Osaka, Japan; 2. NTT WEST Osaka Hospital, Osaka, Japan; 3. Higashiosaka General Hospital, Higashiosaka, Osaka, Japan.